Nox5 regulates vascular smooth muscle cell phenotype

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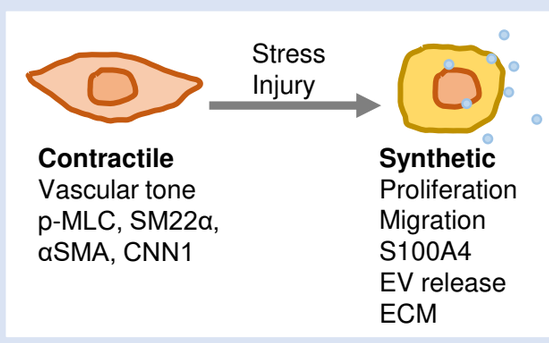
Introduction

VSMC phenotype switching

- Contractile VSMCs regulate vascular tone.
- In response to injury, VSMCs become synthetic [1].
- Synthetic VSMCs lose expression of contractile proteins, proliferate, migrate, secrete extracellular vesicles (EVs) and produce extracellular matrix proteins to repair damage [2, 3].
- Involved in many vascular pathologies, such as atherosclerosis, calcification and aneurysm formation.



Aim

Investigating the molecular mechanisms regulating VSMC phenotype changes.

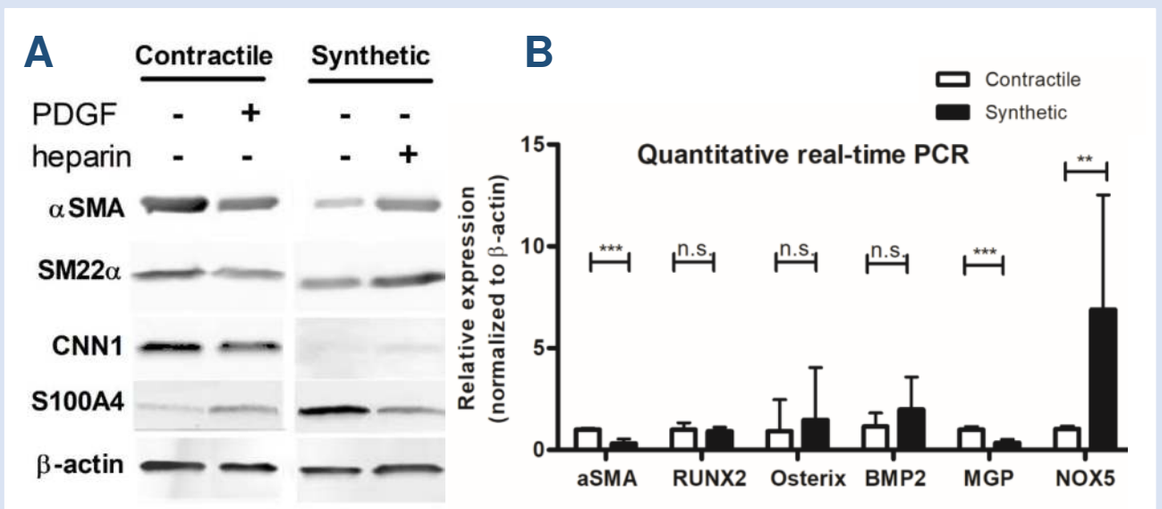
Methods

Human (h) or porcine (p) primary VSMCs were treated with heparin (200 U/ml), PDGF (20 ng/ml), Nox5 or empty lentivirus (MOI=10) or siRNA. Gene expression was analysed by **quantitative real-time PCR (qPCR), a microarray, western blotting and immunohistochemistry.** EVs from cell culture supernatants were quantified using a **bead-capture assay**. Where appropriate, results (n=3) were analysed with t-test or one-way ANOVA and Tukey's *post hoc* test or t-test, (** $p \leq 0.01$; *** $p \leq 0.001$).

Results

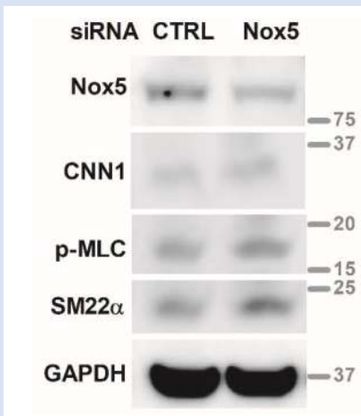
1. Synthetic VSMCs express increased levels of Nox5

We used porcine VSMCs isolated as distinct populations of synthetic or contractile cells [4]. Additionally, these cells can be differentiated to opposite phenotypes using PDGF (promotes synthetic dedifferentiation) and heparin (contractile). **A** – Western blots showing decreased contractile marker expression is associated with synthetic differentiation. Phenotypic switching is reversible. **B** – qPCR analysis of gene expression in contractile vs synthetic VSMCs showing that Nox5 mRNA expression was higher in synthetic VSMCs than contractile.



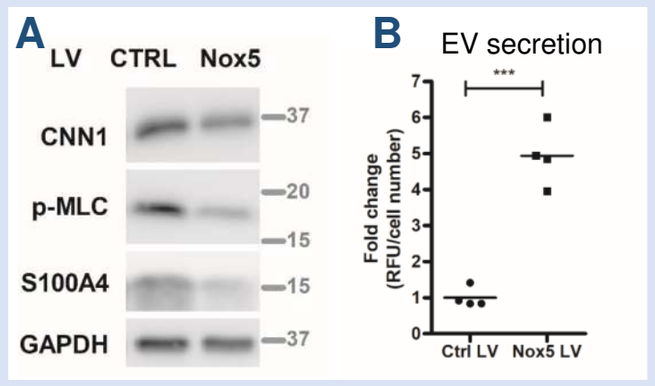
2. Nox5 is involved in phenotype regulation

siRNA knockdown of Nox5 increased expression of contractile markers in hVSMCs.



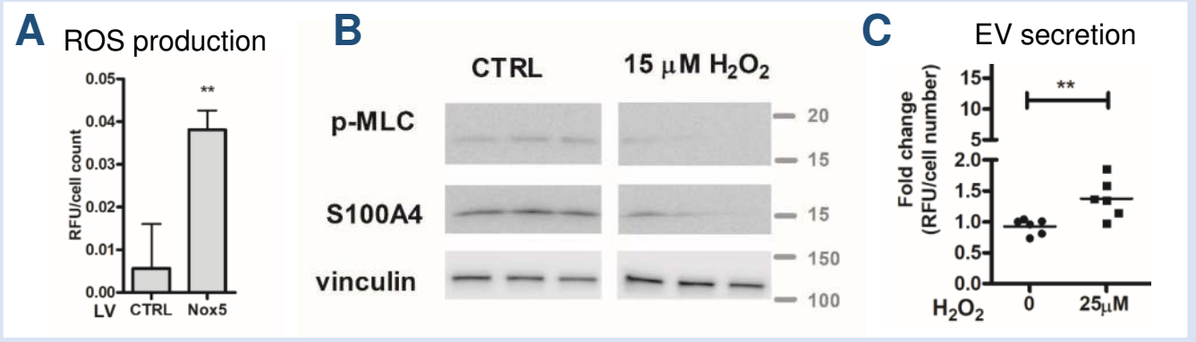
2. Nox5 is involved in phenotype regulation

Lentiviral overexpression of Nox5 in hVSMCs induced hallmarks of the synthetic phenotype: **A** - decreased expression of contractile markers p-MLC and CNN1 measured by western blot and **B -** increased EV release.



4. ROS mediate phenotype changes

Nox5 is a reactive oxygen species (ROS) producing enzyme. **A** - Nox5 overexpression resulted in increased ROS production by hVSMCs **B** - Treating VSMCs with H_2O_2 downregulated expression of p-MLC and **C** - increased EV secretion.

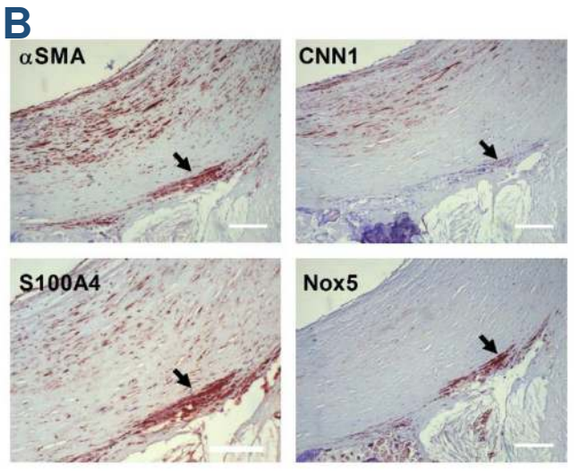


5. Nox5 expression is increased in synthetic VSMCs in human arteries

A - in a transcriptomic analysis Nox5 expression positively correlated with MLC, SMTN, αSMA and negatively correlated with MYH11 and CNN1, suggesting expression in phenotypically modulated VSMCs in human atherosclerotic plaques. **B** - elevated Nox5 expression was observed in VSMCs that lost expression of CNN1 in coronary arteries.

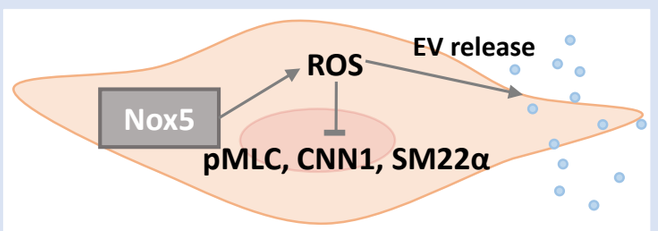
A

	Marker	Pearson r	P value summary
Contractile	MYH11	-0.2691	**
	CNN1	-0.0161	ns
	MYOCD	0.1171	ns
	SM22α	0.1255	ns
	αSMA	0.3943	****
	MYL10	0.4328	****
	SMTN	0.6218	****
	MYL1	0.6692	****
Synthetic	S100A4	-0.313	***
	KLF4	-0.3701	****



Conclusions

Nox5 can induce phenotype changes in VSMCs via ROS. Nox5 is therefore a potential target to modulate vascular remodelling.



References

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